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Investigating Force-Induced Structural Changes in Single Collagen Molecules
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Collagen is a structural protein found in abundance throughout the body, within the extracellular matrix and connective tissues including skin, bone, and tendons. One of the functions of collagen is to maintain tissue structure in the presence of external forces. However the effect of force on the collagen's structure is not clear:1 in the presence of an external force, collagen has been proposed to have one of two opposing structural changes: either overwinding, reducing its cleavage2 or underwinding, increasing its cleavage.3 Using a high-throughput single-molecule stretching instrument, the centrifuge force microscope (CFM),4 we are developing a technique to investigate force-dependent structural changes of collagen under various loading conditions. Here, we report on our progress tethering, enzymatically cleaving and applying a tunable external force to single molecules of collagen. In addition, we present characterization of the instrument and technique accomplished with single DNA molecules.
1.Chang, S.W. and Buehler M. (2014) Molecular biomechanics of collagen molecules. Mat. Today. 17, 70-76.
2.Camp, R., Liles, M., Beale, J., Saeidi, N., Flynn, BP., Moore, E., Murthy, S., and Ruberti, JW. (2011) Molecular Mechanochemistry: Low Force Switch Slows Enzymatic Cleavage of Human Type I Collagen Monomer. JACS, 133, 4073-8.
3.Adhikari, A., Glassey, E. and Dunn, AR. (2012) Conformation Dynamics Accompanying the Proteolytic Degradation of Trimeric Collagen I by Collagenases. JACS, 134, 13259-65.
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Static and Dynamic Effects of Crowders on Mechanical Unfolding of Proteins
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We have analyzed the mechanical unfolding of I-27 in the presence of molecular crowders of different size and concentration. It is widely appreciated that molecular crowders will affect the ground and transition state free energies, and thus provide insights into the folding process. In addition to this thermodynamic effect, however, there is also a substantial effect of the viscosity of the crowded solution on the cantilever. This effect can be quantified and used to correct unfolding forces measured by atomic force spectroscopy.
To account for this effect, the viscous force on the cantilever was calculated, taking into account both the shape of the cantilever as well as the relative velocity of the cantilever respect to the liquid following a procedure described in a previous work. We found that at speeds of 1000 nm/s with a concentration of 300 g/l of 40 kDa Dextran, the drag force can be up to 33% of the total force, while at lower concentrations or smaller molecular sizes, this effect could almost be neglected.
After correction, the unfolding forces increased monotonically for all crowders and concentrations. Unfolding rates at zero-force thus showed a monotonic decrease, both with concentration as well as with the crowder size.
The results argue that crowding can substantially increase the free energy barrier at the transition state creating an even more compact ground state in the presence of crowders.
We modeled the change of free energy using Minton-Ogston's and as well as scaled particle theory, but the crowding effect could not be fully described by either of them, possibly due to the structure of Dextran, especially at higher concentrations.

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Development of a Wireless, Modular Centrifuge Force Microscope for Use in a Commercial Benchtop Centrifuge
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A few years ago, we introduced the Centrifuge Force Microscope (CFM) as a new platform for massively parallel single-molecule manipulation. Our original prototype instrument used a microscope on a rotary stage to generate uniform centrifugal forces, which were applied to thousands of microspheres at once, hundreds of which formed single molecule tethers. Here we present a second generation CFM that is fully integrated into a commercial benchtop centrifuge, further increasing the accessibility of the method and greatly expanding the dynamic force range. We have condensed a complete video microscope to fit into a centrifuge bucket, along with battery power and an onboard computer. The result is a fully wireless ''CFM module'' that can be placed inside a swinging bucket, while still retaining full control of the instrument (even during centrifugation) from a wifi enabled computer. We have demonstrated the unit up to 1000g, which gives a force range of ~70fN to 70pN using 2.8 micron microspheres. Our current device can save images at near USB 2.0 speeds with up to 5-megapixel resolution, and we are working toward improving the bandwidth further. While a number of single-molecule applications exist, one of our primary interests will be to probe the structure and function of various non-coding RNAs.

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Comparison of the Photothermal Efficiency of Different Types of Plasmonic Nanoparticles in vitro and in vivo
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Due to their unique optical properties and biocompatibility gold nanoparticles irradiated with near-infra red (NIR) light are promising candidates for photothermal cancer therapy [Hirsch et al., PNAS 2003, 23, 13549-13554]. In particular, the NIR region is preferable for cancer therapies since it has the lowest absorption and highest penetration depth in biological material. When irradiated, gold nanoparticles efficiently absorb the light and convert it into extremely local and well-controlled heating with temperature increases that easily exceeds 100°C [Bendix et al., ACS Nano 2010, 4, 2256-2262]. We report a comparison study of the capability of NIR-resonant 150 nm silica gold nanoshells as photothermal transducers to that of NIR-off resonant 80 and 150 nm spherical gold nanoparticles in vitro and in vivo. Using a NIR optical trap coupled into a Leica SP5 confocal microscope we investigate the temperature profile as a function of laser power of single irradiated nanoshells and spherical nanoparticles in vitro; this is done using an assay with phospholipid vesicles with a well-characterized phase transition and phase sensitive fluorescent molecules that returns directly the temperature profile of the 3D optically trapped nanoshell or spherical nanoparticle [Kyrsting et al., Nano Letters 2011, 11, 888-892]. The photothermal relationship between laser power and the composition/size of a single particle in vitro are compared to the photothermal efficiency in vivo where the particles were delivered in a mouse tumor model. Nanoparticles were injected directly into the tumor of tumor-bearing mice that were subsequent irradiated with a NIR diode laser. The photothermal efficiency of the nanoshells and spherical nanoparticles in vivo is evaluated by 18F-flourodeoxyglucose positron emission tomography/computed tomography (18F-FDG PET/CT)-based imaging and caliper-based tumor volume shrinkage [Munk Jensen et al., PloS one 2013, 8, e85126].

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Multimodal Imaging Probing Platform Based on Upconverting Rare-Earth Doped Gd2O3 Nanocrystals
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The state-in-art ''multimodalities'' represents the incorporation of individual imaging techniques such as electron, optical and/or magnetic resonance imaging (MRI). The combination enables a comprehensive insight of the interplay within biological objects not only from the molecular level to whole organ, but also from primitive until the ulterior stages of development.
The purposes of our research is to exploit the advantage in resolution limit of cathodoluminescent electron microscopy and the deep penetration emissions through biological tissue in near infrared (NIR) region in order to established a cover wide range multimodal imaging techniques from nano- to meter-scale [1]. The upconversion imaging and magnetic resonance imaging are expected to become potential constitutions of this construction.
Most of imaging techniques use ''energy-matter'' interaction of probes and excitation sources to provide specific details about biological targets. An appropriate multimodal imaging probe is capable of interaction with varied energy source and offers detectable or enhance signals.